Uppsala 1969

IMMUNOGLOBULIN E IN ATOPIC DERMATITIS

By

Sven Öhman

From the Department of Dermatology and the Blood Centre, University Hospital, Uppsala, Sweden

IMMUNOGLOBULIN E IN ATOPIC DERMATITIS

By

Sven Öhman

Digitized by the Internet Archive
in 2026

https://archive.org/details/IA41548912_0034

CONTENTS

I.	General Introduction	5
II.	Serum IgE Levels	7
III.	Allergen-specific IgE in Adults. Comparison between Intradermal and Radioallergosorbent Tests	23
IV.	Allergen-specific IgE in Children	33
V.	Seasonal Variation of IgE Levels in Serum	37
VI.	Skin Reactions to Anti-IgE Antibody	41
VII.	Vascular Skin Reactions in Patients with High and Normal Serum IgE Levels	45
VIII.	Summary	49

UNIV.
LEIDEN
BIBL.

I. GENERAL INTRODUCTION

The term "atopy" was introduced by Coca in 1923 (9). Later he described atopy as "certain clinical forms of human hypersensitiveness that do not occur, so far as is known, in the lower animals, and which are subject to hereditary influence. In this category have been included thus far only asthma and hay fever, but it is generally thought that eczema and certain forms of drug and food idiosyncrasy will eventually be placed with these" (12). Sulzberger and Coca in 1933 (11) suggested the name atopic dermatitis for the characteristic clinical type of dermatitis often associated with atopy.

Though many writers have criticized the term atopy, it has found wide use, and allergic bronchial asthma, allergic rhinoconjunctivitis, atopic dermatitis and certain forms of urticaria are now generally considered as clinical manifestations of the atopic state of the individual. As a convenient designation, the word atopy will here be used in this sense.

Atopic Dermatitis

The disease recognized nowadays as atopic dermatitis, but often referred to as Prurigo Besnier in the Scandinavian literature, was first regarded as a separate entity around 1885 by the French dermatologists Besnier and Brocq. The common association with certain allergic manifestations especially asthma and allergic rhinitis, was noted early.

Atopic dermatitis is a common skin disease and it is estimated that about 3 per cent of all infants suffer from it (43). The basic etiologic factors remain largely obscure. It is well known that many of the patients have a family history of atopy and it has been shown that many of them have an increased liability to reagin formation (10). The reagins or skin-sensitizing antibodies are involved in the pathogenesis of many cases of asthma and allergic rhinitis but their role in eliciting dermatitis has been subject to discussion. Other factors that might be of importance for the pathogenesis of the dermatitis are the dry, irritable skin, a lowered threshold for itching and an increased vasoconstrictor tendency in the skin (36 for ref.). A possible sweat retention might also be involved and psychological factors can aggravate the disease.

Immunoglobulin E

The class of protein to which antibodies belong are nowadays called immunoglobulins. At present five classes of immunoglobulins are known, IgG, IgM, IgA, IgD and IgE. The latest discovered, immunoglobulin E, is only occurring in nanogram quantities in normal serum. This class was first described in 1967 by Ishizaka and by Johansson and Bennich independently (18, 23). It has been shown that the reagins or skin-sensitizing antibodies in serum belong to immunoglobulin E (18, 13). Increased serum levels of IgE have been found in patients with asthma, hay fever and atopic dermatitis (24, 29), and also in patients infested with intestinal parasites (2). With immunofluorescent and autoradiographic techniques, IgE has been shown to be bound to the surface of circulating basophil leucocytes and to the surface of tissue mast cells (20, 16). It is believed that when an allergen reacts with allergen-specific IgE on the surface of the mast cell, this antigen-antibody reaction induces the liberation of vasoactive substances, among others histamine. The action of these compounds accounts for a large part of the symptoms of asthma and allergic rhinitis but their role in atopic dermatitis is still unknown.

The discovery of IgE and the introduction of new, sensitive radio-immunologic techniques have opened up new possibilities for further studies in this field. In the present investigation these techniques have been used to study the role of reagins in patients with atopic dermatitis.

II. SERUM IgE LEVELS

In a previous study of patients who were treated mainly on the wards for severe atopic dermatitis, a high level of IgE was found in about 80 per cent of the cases (29). This motivated a more extended study, which also included patients with milder forms of the disease. The influence of other factors such as asthma, allergic rhinitis, severity of dermatitis, etc., was also investigated.

Material and Methods

Patients. During the first half of 1969 a total of 313 patients with a diagnosis of atopic dermatitis visited the outpatient clinic of the Department of Dermatology, University Hospital, Uppsala. They were all requested to take part in the present investigation. After re-examination and -interview, 58 patients were excluded from the study as the diagnosis was questionable. Most of the excluded patients had had an atopic dermatitis in childhood and were now, as adults, experiencing a hand eczema which was most likely of toxic origin. The criteria used for the diagnosis of atopic dermatitis were the same as given by Pillsbury *et al.* (35).

Of the remaining 255 patients, 221 (87 per cent attendance) took part in the study. They included 64 boys and 64 girls under 15 years of age (89 per cent attendance). Their age distribution is shown in Figure 1. The adult group, those over 15 years of age, consisted of 35 men and 58 women (84 per cent attendance). Their age distribution is shown in Figure 2. The majority of those who did not take part were unreachable due to change of address.

A detailed case history was obtained from all the patients in which special attention was given to allergic manifestations such as asthma, allergic rhinitis and urticaria. The severity of their atopic dermatitis was also graded on a scale 1 to 3.

Grade 1 included patients with a *mild* dermatitis, mostly in the form of slightly eczematous changes in the elbow and knee flexures, wrists or ankles.

Grade 2 was a *moderate* dermatitis with more pronounced eczematous

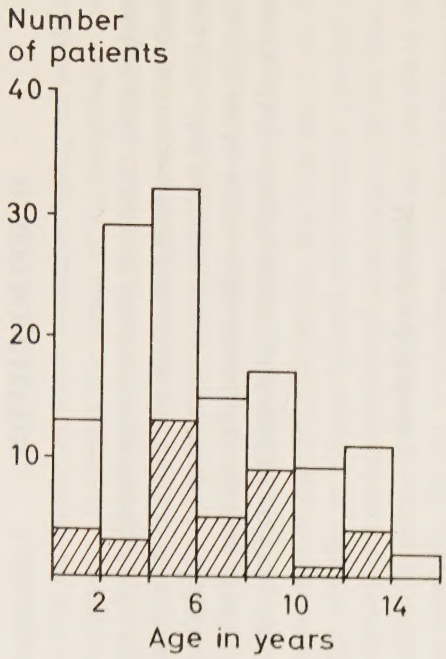


Fig. 1. Age distribution in children with atopic deramtitis.
□ = "pure" type of dermatitis; ▨ = "mixed" type of dermatitis.

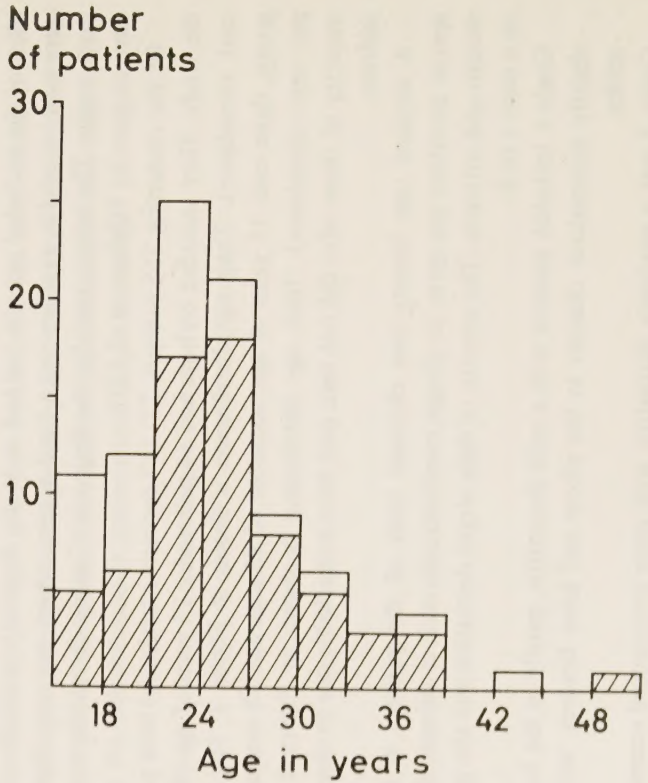


Fig. 2. Age distribution in adults with atopic dermatitis.
□ = "pure" type of dermatitis; ▨ = "mixed" type of dermatitis.

changes in the flexures and also a few eczematous patches elsewhere on the limbs, trunk or head.

Grade 3 consisted of patients with a *severe* dermatitis which was often badly excoriated, with pronounced eczematous changes in most flexures on the limbs and also eczematous changes over large areas of the body.

In order to minimize any possible seasonal influence on the serum level of IgE, nearly all of the patients were studied during the latter part of 1969. They were all examined by the author.

Capillary blood samples were taken from the children and, in the adults, the determinations were carried out on venous blood. The serum was separated after 2 to 3 hours at room temperature and stored at $-20^{\circ}C$ until analyzed.

Determination of IgE. The IgE concentrations were determined according to the radio-immunosorbent-technique (RIST) of Wide & Porath (46) as applied to IgE determinations by Johansson *et al.* (27). The principle of this method is that the concentration of IgE in a serum sample is measured by its capacity to inhibit the binding of ^{125}I-labelled IgE to anti-Fc IgE antibodies coupled to an insoluble matrix, a Sephadex particle, as compared to a standard solution of IgE. The coupling to the cross-linked dextran in the Sephadex particle is accomplished by activation with cyanogen bromide (1). The uptake of labelled IgE, measured in terms of radioactivity, is inversely related to the amount of IgE in the sample. The inhibition technique used is based on antibodies against myeloma protein ND and I^{125} labelled IgND. This carries with it a certain risk of idiotypic antigen influence, i.e., of measuring IgND instead of IgE. We have tried to minimize the influence of these factors by using anti-Fc ND, considering the fact that the idiotypic antigens are located on the Fab part of the molecule. Absorption with Fab (ND) fragments is also done.

For a RIST experiment 0.2 ml of a suitable dilution of the sample (about 1:10) and 0.1 ml of labelled IgE, in a dilution corresponding to about 10-15,000 counts per minute (cpm), were mixed with 1 ml of buffered saline containing a suspension of Sephadex particles coated with a rabbit anti-IgE, 10 to 20 μg of the Sephadex-antiserum-complex per ml. The 0.05 M phosphate buffered saline, pH 7.4, contained 1% Tween 20 and, to avoid unspecific reactions, the following substances were added to a final concentration: IgG 1 μg/ml; Fab fragments of E myeloma protein ND 0.1 μg/ml;

Bence-Jones protein type L from E myeloma case ND 0.1 μg/ml; normal rabbit gamma globulin 10 μg/ml. The normal rabbit gamma globulin was added to avoid any possible influence of heterophile antibodies in the patient's serum. These antibodies, which occur in a high frequency (26), may react with the anti-IgE on the particles and thereby sterically interfere with the IgE-anti-IgE reaction, thus giving rise to false high IgE levels.

After incubation overnight (16 hours) at room temperature, the excess of the labelled IgE was washed away with a saline containing 1% Tween 20 by centrifuging at 3,000 rpm. Finally the radioactivity remaining on the particles was determined in a gamma scintillation counter and the IgE concentration in the sample was calculated from a standard curve. One serum sample with a high IgE concentration (6,000 ng/ml) and one with a low (70 ng/ml) were always included in every run. All the venous samples were analyzed in duplicate. IgE concentrations as low as 5 ng/ml could be measured. The error of the method calculated as the standard deviation (SD) for duplicate analysis of samples is about 15%. Using this method the mean value of IgE in serum from healthy adults was found to be 248 ng/ml with a 95 per cent confidence interval of 60 to 1,000 ng/ml (22).

There have been some problems with the standardizations of the IgE levels and we have reasons to believe that the normal values published previously are somewhat too high (22, 4, 7). With improved technique, we seem to be better able to discriminate between the very low values.

After the present investigation was completed the Immunoglobulin Reference Centre of WHO has distributed reference sera to facilitate comparisons between different laboratories. It is also recommended that the IgE values should be given in units instead of nanogram per ml. Analyses of WHO sera which are now under way will make it possible to transform earlier reported nanogram values to WHO standard units.

Statistical calculations. The frequency distribution of serum IgE values in healthy persons is skewed to the right when arithmetic values are used but rather normally distributed when using logarithmic values. The calculations, therefore, have been done using logarithmically transformed values. In children the IgE level is known to rise slowly with age (4); as can be seen in Figure 4 where the regression line for healthy children (log IgE on age) is superimposed on all the actual values for the children in this study. As a consequence of this age-dependent increase of the IgE concentration, we

have, for statistical reasons, preferred to express the IgE value in children as a "deviation from the expected mean value for the age" transformed logarithmically to base 10. This is called the residual value (R.V.), see Figure 3. The expected mean value for the age has been calculated from the formula $^{10}\log IgE = 2.10016 - 0.0223 \times$ age in years (4).

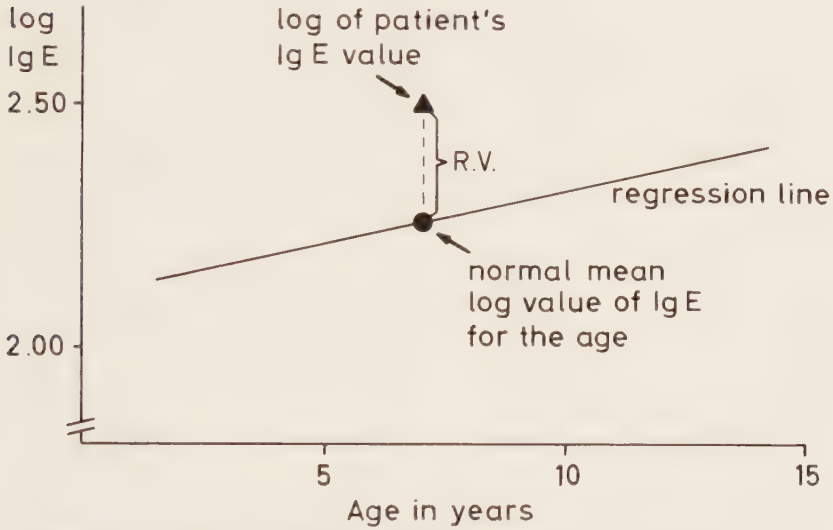


Fig. 3. Calculation principle of residual value (R.V.) of log IgE in children.

Results

Atopic dermatitis and combinations with asthma and allergic rhinitis

Children. Figure 4 shows the individual IgE values in the children with atopic dermatitis. The geometric mean value and mean of residual values (R.V.) for the entire group are given in Table I. Here, a division has also been made into patients with or without symptoms of bronchial asthma or allergic rhinoconjunctivitis. In the following, patients with only an atopic dermatitis will be referred to as "pure". Those with symptoms of asthma and/or allergic rhinitis in addition to their dermatitis will be referred to as "mixed".

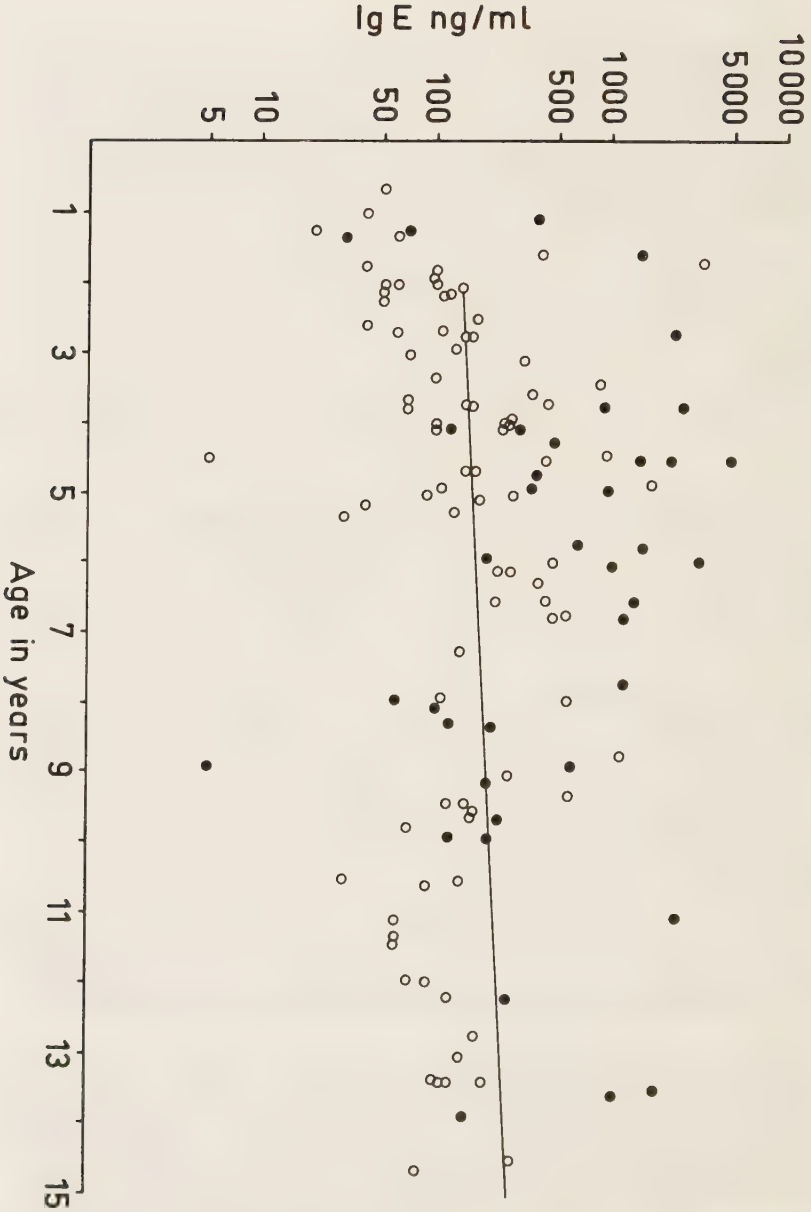


Fig. 4. Serum IgE values in 128 children with atopic dermatitis. o, children with only dermatitis ("pure"); ●, children with dermatitis + asthma and/or allergic rhinitis ("mixed"). The regression line used for calculation is superimposed.

TABLE I. Mean values of serum IgE in children with atopic dermatitis.

	Combinations of atopy							
	during lifetime				during the last three years			
Atopy coexistent with dermatitis	No. of patients	R.V.* Mean	SD	Geometric mean** of IgE ng/ml	No. of patients	R.V.* Mean	SD	Geometric mean** of IgE ng/ml
None ("pure")	89	–0.0835	0.4421	141	94	–0.0810	0.4866	143
Asthma and/ or allergic rhinitis ("mixed")	39	+0.4071	0.6449	452	34	+0.4722	0.5471	520
Asthma	19	+0.5468	0.7187	585	14	+0.7547	0.4105	904
Allergic rhinitis	8	+0.1545	0.5203	269	8	+0.1545	0.5203	269
Asthma and allergic rhinitis	12	+0.3543	0.5800	424	12	+0.3543	0.5800	424
Total	128	+0.0660	0.5579	201	128	+0.0660	0.5579	201

* R.V. = residual value of IgE, expressed in ^{10}log. The residual value of IgE is the difference between the patient's ^{10}log IgE value and the normal mean value of ^{10}log IgE for the age, measured in ng/ml.
** Not corrected for age.

The children with "pure" atopic dermatitis had a mean R.V. of IgE in serum slightly below zero. From our definition of R.V. follows that the normal mean of IgE (4) corresponds to a R.V. of zero.

In children who had a "mixed" atopic dermatitis, the mean R.V. of IgE was raised above zero ($p < 0.001$) and it differed significantly ($p < 0.001$) from the mean of children with a "pure" atopic dermatitis. Patients with atopic dermatitis and asthma had the highest mean, followed by the group with atopic dermatitis and both asthma and allergic rhinitis, and finally the children with atopic dermatitis and only allergic rhinitis. An analysis of variance showed no significant difference between these last three subgroups.

To the right in Table I the grouping of the results is designed to show whether the child had had any symptoms of asthma or allergic rhinitis during the last three years. Of the five children who no longer had asthma, four had normal IgE values, i.e. R.V. close to zero. The mean R.V. now becomes somewhat larger for the "mixed" group. The same is true for the subgroup with dermatitis and asthma, which now has a significantly higher value ($p < 0.05$) than the groups with allergic rhinitis alone or in combination with asthma.

Adults. In Figure 5 the individual IgE values are shown for the adult patients. In Table II the geometric means of the actual IgE values are given, expressed as nanograms per ml. The mean IgE value of the patients who in addition to their dermatitis had a history of asthma and/or allergic rhinitis ("mixed" group) was higher ($p < 0.01$) than in the group with "pure" dermatitis. The highest mean was recorded in patients with both asthma and allergic rhinitis.

Essentially the same findings were obtained when the patients were grouped according to the symptoms they had displayed during the last three years (right part of Table II).

History of urticaria

A history of urticaria some time in life was obtained in 7 per cent of the adults and 9 per cent of the children with a "pure" atopic dermatitis. Only one of the eight children with a "pure" dermatitis and a previous history of urticaria had an elevated serum IgE value. His RAST-analyses were negative (see Part IV, table IX, patient No. 2). In the adults both patients with a "pure" dermatitis and a history of urticaria had normal IgE levels in serum.

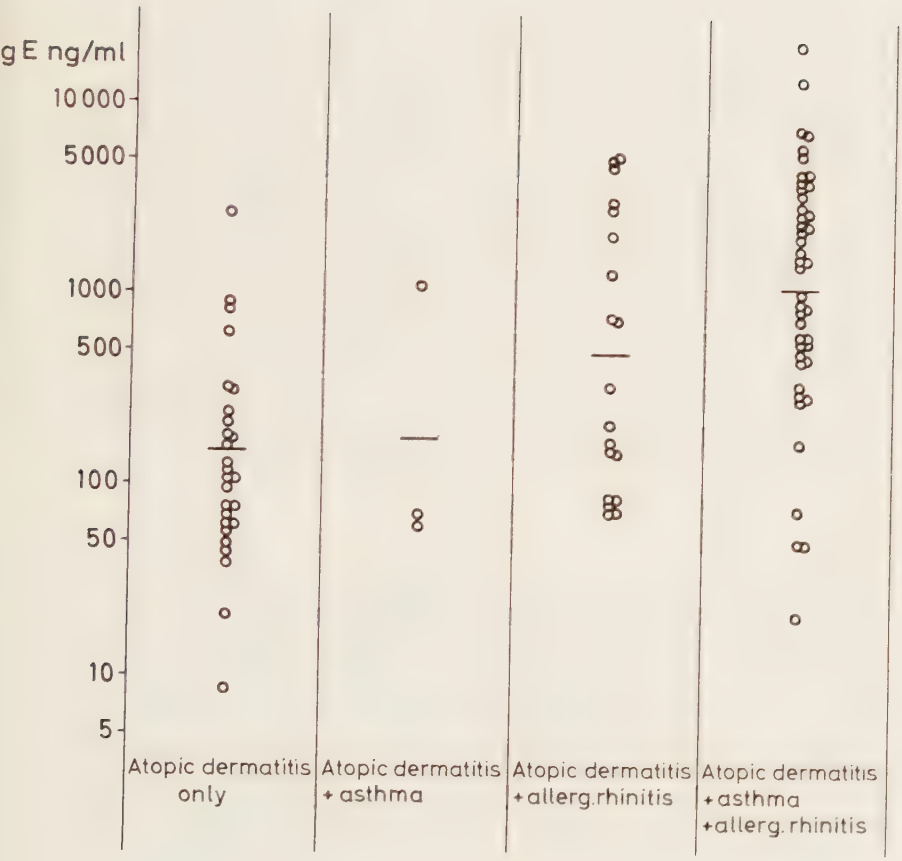


Fig. 5. Serum IgE values in 93 adults with only atopic dermatitis and combinations with asthma and/or allergic rhinitis.

TABLE II. Mean values of serum IgE in adults with atopic dermatitis.

	Combinations of atopy							
	during lifetime				during the last three years			
Atopy coexistent with dermatitis	No. of patients	^{10}log IgE Mean	SD	Geometric mean of IgE ng/ml	No. of patients	^{10}log IgE Mean	SD	Geometric mean of IgE ng/ml
None ("pure")	27	2.1573	0.4792	144	30	2.1800	0.5112	151
Asthma and/ or allergic rhinitis ("mixed")	66	2.9190	0.7055	830	63	2.9445	0.6954	880
Asthma	3	2.2321	0.7227	171	4	2.5639	0.5475	366
Allergic rhinitis	19	2.6952	0.7086	496	27	2.9611	0.7128	914
Asthma and allergic rhinitis	44	3.0625	0.6646	1154	32	2.9780	0.7014	951
Total	93	2.6979	0.7330	499	93	2.6979	0.7330	499

In the "mixed" group urticaria had occurred in 30 per cent of the adults and 26 per cent of the children. Most patients only had had a few episodes of urticaria.

Severity of atopic dermatitis

Children. When a comparison was made on the basis of severity of the atopic dermatitis, it was found that children with a mild dermatitis had a mean R.V. of IgE very close to zero (Table III). When pooled together, the groups with a moderate or a severe dermatitis had a significantly higher mean R.V. ($p < 0.05$) than those with a mild dermatitis.

To avoid the influence of asthma and allergic rhinitis, the same grading system was used for the children with a "pure" atopic dermatitis. As can be seen from the right part of Table III there are no significant differences between the groups with mild, moderate or severe dermatitis.

Adults. As can be seen from Table IV, the mean IgE value increased considerably with increasing severity of the atopic dermatitis when both "pure" and "mixed" patients were considered. The values differed significantly ($p < 0.01$). When the same analyses were made for only those with a "pure" dermatitis, the groups were too small to show a possible difference, but, when those with a moderate or severe dermatitis in the "pure" group were pooled together, they had a significantly higher ($p < 0.01$) mean IgE value than those with a mild dermatitis.

Food intolerance

Forty-three per cent of the children and 44 per cent of the adults stated that their dermatitis was aggravated by some type of food or beverage. In children the mean R.V. for the groups with and without stated food intolerance did not differ significantly. This was true for both "pure" and "mixed" groups and the entire group of children (Table V).

In the "mixed" group of adults, a higher mean IgE value ($p < 0.01$) was found in patients without food intolerance, as compared to those with food intolerance (Table V). No such difference could be seen within the "pure" group of adults.

Heredity

Seventy-eight per cent of the children and 66 per cent of the adults had relatives of the first and second degree with some kind of atopy. When the

mean IgE values were compared, no significant difference could be found between those with a family history and those without, (Table V).

Age of onset

No correlation between reported age of onset and the actual IgE values was found.

In both children and adults, those with a stated food intolerance had a significantly lower median age of onset ($p < 0.01$) compared to those without food intolerance.

The children with a moderate or severe type of dermatitis also had a significantly lower median age of onset ($p < 0.05$), when compared to those with a mild form. In the adult group no such differences could be found.

There was no significant difference in the median age of onset between patients with and without a hereditary background.

Discussion

For a study of this type, it is a problem to find a group of patients which represents a true cross section of the disease. Some patients with minor symptoms will never see the doctor. Other patients are seen by doctors outside the dermatology clinic; often because they are under care for other major diseases. Since there are no dermatologists in the Uppsala area except at our hospital, a large part of the patients with atopic dermatitis are seen at our outpatient clinic. The patients studied might therefore be considered as fairly representative of the actual panorama of atopic dermatitis.

Thirty per cent of the children belonged to the "mixed" group, as compared to 71 per cent of the adults. This could be explained by a selection if the pediatricians also treat the dermatitis of the patients with asthma and allergic rhinitis. Another possibility is that an increased number of children will develop symptoms of asthma or allergic rhinitis later in life (15). Still another possibility could be that the presence of asthma or allergic rhinitis would lead to a more persistent dermatitis if the patient is prone to have skin changes.

The IgE values in healthy subjects with no history of allergy have been estimated by Johansson and Berg (22, 4). The same technique has been used in the present study, but since the IgE determinations were not made at the

TABLE III. Mean values of serum IgE in children with regard to severity of atopic dermatitis.

Severity of atopic dermatitis	All children				Children with "pure" atopic dermatitis			
	No. of patients	R.V.* Mean	SD	Geometric mean** of IgE ng/ml	No. of patients	R.V.* Mean	SD	Geometric mean** of IgE ng/ml
Mild	86	−0.0087	0.5220	171	69	−0.1117	0.4408	134
Moderate	29	+0.2264	0.5610	290	17	−0.0004	0.4705	161
Severe	13	+0.2020	0.7129	262	3	+0.0931	0.3040	217
Total	128				89			

* R.V. = residual value of IgE, expressed in ^{10}log. The residual value of IgE is the difference between the patient's ^{10}log IgE value and the normal mean value of ^{10}log IgE for the age, measured in ng/ml.
** Not corrected for age.

TABLE IV. Mean values of serum IgE in adults with regard to severity of atopic dermatitis.

Severity of atopic dermatitis	All adults				Adults with "pure" atopic dermatitis			
	No. of patients	^{10}log IgE Mean	SD	Geometric mean of IgE ng/ml	No. of patients	^{10}log IgE Mean	SD	Geometric mean of IgE ng/ml
Mild	55	2.4166	0.6520	261	20	2.0481	0.4720	112
Moderate	18	2.8342	0.6199	683	4	2.3754	0.4048	237
Severe	20	3.3487	0.5977	2232	3	2.5948	0.3492	393
Total	93				27			

TABLE V. Mean values of serum IgE with regard to food intolerance and hereditary background of atopy.

	Children			Adults			
	No. of patients	R.V.* Mean	SD	No. of patients	^{10}log IgE Mean	SD	Geometric mean of IgE ng/ml
Food intolerance							
"pure" group	34	−0.0267	0.4593	7	2.3994	0.6309	251
"mixed" group	21	+0.5378	0.5769	34	2.7011	0.7043	503
total	55	+0.1888	0.5734	41	2.6496	0.6944	446
No food intolerance							
"pure" group	55	−0.1187	0.4317	20	2.0726	0.3993	118
"mixed" group	18	+0.2546	0.7018	32	3.1505	0.6388	1414
total	73	+0.0266	0.5313	52	2.7359	0.7667	544
Hereditary background of atopy							
"pure" group	70	−0.0752	0.4232	17	2.2220	0.5168	167
"mixed" group	30	+0.3653	0.6675	44	2.8323	0.7218	680
total	100	+0.0570	0.5445	61	2.6622	0.7216	459
No hereditary background of atopy							
"pure" group	19	−0.1143	0.5177	10	2.0474	0.4091	112
"mixed" group	9	+0.5462	0.5766	22	3.0924	0.6531	1237
total	28	+0.0980	0.6131	32	2.7658	0.7614	583

* R.V. = residual value of IgE, expressed in ^{10}log. The residual value of IgE is the difference between the patient's ^{10}log IgE value and the normal mean value of ^{10}log IgE for the age, measured in ng/ml.

same time as in our patients, we have been careful when drawing conclusions from comparisons with earlier reported IgE levels in healthy subjects. We have reasons to believe that the technique now has improved when estimating IgE values below 100 ng/ml. Since our calculations are based on the geometric means, a decrease of 20 to 40 ng/ml at this level will have a comparatively great influence on the mean. This might explain why the mean IgE level of our adult patients with "pure" dermatitis, i.e. without asthma and allergic rhinitis, is slightly lower than the previously given normal geometric mean. For the higher IgE values the method seems less sensitive to minor methodological changes.

The mean IgE value for both the children and the adults in the "mixed" group, those with asthma and/or allergic rhinitis in addition to their dermatitis, was found to be higher than for those in the "pure" group and also higher than the earlier reported normal mean values for healthy children and adults. Even if a comparison with a "normal" IgE value, as mentioned earlier, might be questionable for values lower than the "normal", it is likely that, for values higher than "normal", a found difference might be even more significant.

Previous studies have shown increased serum IgE levels in patients with asthma and allergic rhinitis (21, 5). It is therefore possible to link the common finding of a higher IgE value in the "mixed" group to the concomitant symptoms of asthma and/or allergic rhinitis. The total IgE level can roughly be regarded as the sum of all reagins. It is well known that, when reagins react with their antigens, symptoms of asthma or allergic rhinitis may be provoked. It is also possible, however, to have a few circulating allergen-specific reagins which will not be detected as an increased IgE value, and all allergens might not stimulate the IgE production to the same extent. Also, we know very little about to what extent the circulating IgE might reflect local concentrations in various organs, e.g. the skin. The low IgE values found in patients with a "pure" dermatitis therefore gives no clue to whether the reagins are involved in the etiology of the dermatitis.

In many of the patients with a previous history of urticaria this dated far back and was often a single episode. We have not investigated the IgE level in our patients during an acute attack. However, in patients with acute or chronic urticaria without atopic dermatitis, asthma or allergic rhinitis the IgE level was not increased (29). This and our own findings do not lend any support to the possibility that a history of urticaria would influence the IgE level.

When studying the severity of the atopic dermatitis it was found that, in adults and the "mixed" group of children, those with more extensive involvment of the skin also had a higher mean IgE value than those with a milder type of dermatitis. This would indicate that the reagins actually are involved in eliciting the skin changes. One must here, however, take into consideration that a grading of the severity of atopic dermatitis is a crude method with a certain amount of subjectivity included and influenced by treatment and sudden flare-ups. In a recently published study of 23 patients with varying degrees of atopic dermatitis, Ogawa *et al.* (33) also found a correlation between serum IgE levels and the severity of the dermatitis.

The fact that in a previous study (29) no correlation was found between the severity of dermatitis and the IgE level could probably depend on material consisting mainly of patients treated on the wards for severe skin changes. Under such circumstances a relationship would be hard to detect.

In our children with asthma and atopic dermatitis the mean IgE value was higher than in the other "mixed" groups. In a previous study of children with atopic diseases (5), the same tendency was also found. One explanation could be that the presence of dermatitis might in some way enhance the reagin response in these children. Why the children who have asthma and rhinitis in addition to their dermatitis have a lower IgE is uncertain, but, since patients with only allergic rhinitis have a less elevated mean IgE level, the rhinitis could be the dominating symptom in this group. The finding in children could not be reproduced in adults, possibly because we only had four patients in the group with dermatitis and asthma, and their asthma attacks were few and minor during the last years.

Many authors agree that there are cases of "food allergy" but that these are rare after 3 to 5 years of age. Others claim that "gastrointestinal allergy is a diagnosis frequently entertained, occasionally evaluated, and rarely established" (17). There is a certain amount of uncertainty in our figures for food intolerance due to the lack of a reliable provocation test, especially when measuring the effect on the skin. The fact that some patients and parents are more observant than others must also be considered. With no significant difference found in mean IgE between the children with and without food intolerance, we could not elucidate this problem further. It is of interest, however, to note that the median age of onset was lower for those with a stated food intolerance when compared to those without. This was found in both children and adults and can be due to that food allergens

often are among the first the child will encounter.

The lower mean IgE value in adults with food intolerance, compared to those without, is difficult to explain. One can only speculate that adult patients with food intolerance might for many years have avoided the antigenic foods and therefore have a lower IgE value than those without food restrictions.

III. ALLERGEN-SPECIFIC IgE IN ADULTS
Comparison between Intradermal and Radioallergosorbent Tests

For diagnosing hypersensitivity in atopic patients scratch and intradermal tests with various allergens have been used in clinical practice. With the radioallergosorbent test (RAST) of Wide *et al.* (45) a new and *in vitro* method was introduced to determine allergen-specific IgE antibodies. This technique has been used to study circulating reagins in patients with asthma and allergic rhinitis, and comparisons have been made between RAST, intradermal tests and provocation tests in these groups of patients (45, 40, 14, 41, 3).

In the present study the results obtained with intradermal tests and RAST using common allergens have been compared in patients with atopic dermatitis who had elevated and normal IgE values. The circulating eosinophil and basophil leucocytes are often claimed to be involved in allergic reactions and the number of them was also studied in relation to the serum IgE levels.

Material and Methods

Patients. I. Twenty patients with a high IgE level (> 1,000 ng/ml) in serum, 10 males and 10 females, aged 17 to 35 years. They were selected from the material reported in Part II. The patients with IgE values above 1,000 ng/ml were given numbers at random and the first twenty were asked to take part. When this was impossible, due to geographic reasons, working conditions, etc., the next number above twenty was picked. Nineteen of the twenty patients also had had symptoms of asthma and/or allergic rhinitis during the last three years. Eight patients had a dermatitis graded as mild, seven as moderate and five as severe (see Part II).

II. Sixteen patients with an IgE level in serum within normal limits (125 to 500 ng/ml), 4 males and 12 females, aged 16 to 37 years. They were selected from the earlier study, as described above. Seven of the sixteen patients had had symptoms of asthma and/or allergic rhinitis during the last three years. In nine patients the dermatitis was classified as mild, in five as

moderate and in two as severe.

The venous blood samples were drawn immediately before the intracutaneous tests. The serum was separated after 2 to 3 hours at room temperature and stored at −20°C until analyzed for total IgE and specific reagins.

Allergen extracts. The following 13 commercially available allergen extracts were used in the RAST and skin tests: dander from horse, cow (Vitrum AB, Sweden), dog and cat (Dome Chemicals Inc. New York, N.Y.); pollens from birch, timothy, dandelion and ox-eye daisy (Vitrum AB); wool and house dust (Dome Inc.); unboiled egg yolk, unboiled milk and fish (Vitrum AB). The same batches of allergen extracts were used in both the RAST and skin tests. The only exception to this was birch pollen, but here the two extracts used were compared with the RAST-system (28) and found to be of a very similar quality.

In the intradermal tests the following types and numbers of allergen extracts were also included: dust (1), excelsior (1), mold mixture (1), foods (9), feathers (1), pollens (3) and plants (7) (Vitrum AB, Sweden).

Intradermal tests. 0.1 ml of each extract was injected intradermally in the back of the patient. The standard dilutions used were 1:1,000 of extracts from Vitrum AB, and 100 PNU of extracts from Dome Inc. Coca's solution was used as a control and histamine 1:10,000 as a reference standard. The tests were read after 20 minutes and were considered positive when the sum of two diameters of the wheal, measured at right angles, were 20 mm or more. A sum of 30 to 40 mm was graded two plus, and 40 to 50 mm as three plus, etc. The histamine reaction could be regarded as a three plus reaction in all patients except four. When correction was made for this, only two of the test results listed in Table VI had to be changed.

IgE and RAST analyses. The IgE determinations were carried out according to the technique described in Part II. The reaginic antibodies of IgE type against the 13 allergens mentioned above were estimated using the radioallergosorbent technique (RAST) of Wide *et al.* (45). The principle of this technique is the following: using a cyanogen-bromide-activation technique (1) the allergen is coupled to an insoluble matrix of cellulose particles (Microcristalline, Merck). Antibodies of the IgE and other classes in the patient's serum are then allowed to combine with the allergen on the partic-

les and the IgE antibodies are thereafter detected by their ability to bind ^{125}I-labelled antibodies against IgE.

For a determination 0.5 ml of a suspension of the allergen-coated particles of an appropriate concentration (see below) and 50 µl of the serum sample were incubated in stoppered, disposable, plastic tubes under continuous vertical tumbling at 25°C for 18 hours. After 4 washings with 0.15M NaCl containing 1% Tween, 0.1 ml ^{125}I-labelled anti-IgE corresponding to approximately 60,000 counts per minute and containing 0.5 mg/ml of normal rabbit gamma globulin was added. The mixture was incubated at 25°C for 18 hours. The excess of labelled anti-IgE was washed away and the radioactivity bound to the particles was measured in a gamma scintillation detector. Under optimal conditions, approximately 60% of the added radioactivity was bound to the particles. The background activity, using normal serum or buffer controls, was of the order of 100 to 200 counts per minute (0.2 to 0.3% of the added radioactivity).

The standard serum used for quantitative estimation in RAST was obtained from an untreated, birch-pollen sensitive patient with a high concentration of IgE antibodies. This standard serum was divided in aliquots stored at −20°C. The concentration of reagins to any allergen is then given in per mille of the standard serum used, as described by Johansson *et al.* (25). For convenience, a scale of 1 to 7 is often used where 1 means a value of 3-5.6 per mille and 7 a value of more than 1,000 per mille. Since the allergen content in the extracts varies considerably both on a quantitative and qualitative basis, the results cannot, as a rule, be compared between different allergens. But for each allergen the results in different individuals can be compared, as well as in different serum samples from the same individual.

Blood cells. The number of white blood cells (WBC) per mm^3 and circulating eosinophil leucocytes per mm^3 was determined with the routine laboratory techniques, using venous blood collected in EDTA-containing test tubes. The basophil leucocytes in blood were determined as described by Shelley and Juhlin (38).

Results

The skin tests with the 36 allergens showed a higher number of positive reactions in the patients with a high IgE level. This group had an average of 9.2 positive skin tests (range 2 to 19), occurring in all 20 patients. Those with a normal IgE level had an average of 4.4 positive skin tests (range 0 to 12), occurring in 12 of 16 individuals. The number of strongly positive reactions (three to four plus) was also larger in the patients with a high IgE in serum (25, occurring in 13 of 20 patients) as compared to those with normal IgE values (2, occurring in 2 of 16 patients). Most of the strongly positive reactions were encountered with pollen.

Patients with a high IgE level also had a higher number of positive RAST reactions. They had an average of 4.6 positive reactions (in 17 of 20 patients), compared to an average of 1.3 (in 11 of 16 patients) for those with a normal IgE. In the same manner the number of strongly positive RAST-reactions (RAST 5 to 7) was larger in the patients with high IgE, 18 (in 8 of 20 patients) versus 2 (in 2 of 16 patients) in the group with normal IgE.

In Table VI the results are listed from the comparative study of intradermal tests and RAST on thirteen allergens. The results from both the group with high and normal IgE values have been pooled together.

When studying the sum of all tests, they agreed, that is both were either positive or negative, in 75 per cent of the 468 test-pairs.

In 79 (17 per cent) of the test-pairs the skin test was positive, but the RAST negative. Three of these positive skin tests were graded three plus (+++), one was for horse dander, one for oxe-eye daisy and one for dandelion pollen (Table VI). Both pollen reactions were recorded in a patient with severe dermatitis. He had positive RAST reactions to several animal danders, to birch pollen, to milk and fish and a history of allergic rhinitis, at least when exposed to birch pollen. The strongly positive skin test to horse occurred in a patient, who had not been in contact with horses for many years.

The RAST reaction was negative in 25 tests where the intradermal tests were graded as two plus. The discrepancies were mostly seen with extracts from house dust, cow, horse and timothy and occurred to the same extent in patients with high and normal serum IgE values.

Of the positive intradermal tests graded as one plus, 51 had a correspond-

TABLE VI. Comparison of RAST and intradermal test results in all studied patients with atopic dermatitis.

	STRENGTH OF INTRADERMAL TEST REACTION															
	0				+				++				⩾ +++			
	R	A	S	T	R	A	S	T	R	A	S	T	R	A	S	T
Allergen	0	1	2–4	5–7	0	1	2–4	5–7	0	1	2–4	5–7	0	1	2–4	5–7
Horse	12	2	2	0	7	2	2	0	4	0	1	0	1	0	2	1
Dog	25	1	2	0	1	3	1	0	0	0	2	1	0	0	0	0
Cat	17	1	3	0	3	1	4	0	1	1	4	1	0	0	0	0
Cow	10	1	0	0	15	0	3	0	6	0	0	0	0	0	0	1
Wool	33	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0
Birch	20	1	2	0	1	0	1	0	0	0	4	2	0	0	1	4
Timothy	20	4	0	0	4	1	0	0	4	0	0	0	0	0	3	0
Ox-eye daisy	19	1	0	0	6	0	0	0	2	1	1	0	1	0	2	3
Dandelion	23	1	1	0	3	0	2	0	0	0	2	2	1	0	0	1
House dust	8	0	1	0	9	0	0	0	7	0	8	3	0	0	0	0
Egg yolk	33	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0
Milk	26	1	6	0	1	0	1	0	1	0	0	0	0	0	0	0
Fish	30	2	3	0	1	0	0	0	0	0	0	0	0	0	0	0
Total	276	15	23	0	51	7	15	0	25	2	23	10	3	0	8	10

ing RAST reaction which was negative. This means that more than two-thirds of the one plus skin tests had a negative RAST result. The finding was especially common with extracts from cow, house dust, horse and ox-eye daisy. For house dust, the discrepancy was as common in the group with high as with normal serum IgE. For the other extracts it was more commonly seen in the patients with a high IgE level.

The finding of a positive RAST reaction, where the corresponding intradermal test was negative, occurred in 15 + 23 tests = 38 tests. The isolated RAST-positive reaction was never stronger than 3. It was mostly encountered for the food extracts used and also for some of the animal danders and most common in the group with high serum IgE levels. Three patients with a high IgE value accounted for 15 of the 23 negative intradermal reactions in the group with RAST 2 to 4.

The nine patients with a "pure" atopic dermatitis had altogether 9 positive RAST reactions of strength 1 to 3, occurring in 5 patients. Four of the reactions were positive to pollen, two to animal dander, two to milk and one to house dust. The intradermal test in these 9 patients showed 14 one plus reactions and 9 two plus reactions. None of the one plus reactions had a corresponding positive RAST. In one RAST of strength 3 the intradermal test was two plus and RAST strength 2 had a positive intradermal reaction in 3 of 5 tests.

Comparison of test results and history of allergy. No provocation tests were done, but all the patients were questioned on several occasions about allergic manifestations when they were exposed to the allergens which had given positive test reactions. There was agreement with the history of illness in 48 per cent of the positive skin tests and in 51 per cent of the RAST-positive reactions. In 13 per cent of the patients with negative intradermal test and in 15 per cent with negative RAST, the patients gave a history of intolerance when exposed to an allergen. Further details are given in Tables VII and VIII.

Eosinophil and basophil leucocytes. The mean value of eosinophil leucocytes in the peripheral blood was higher ($p < 0.01$) in patients with a high serum IgE level (479/mm^3, SD. 395) as compared to those with a normal serum IgE level (154/mm^3, SD 115). In the patients with high serum IgE values, there was a slight correlation between IgE value and the number of

circulating eosinophils (correlation coefficient 0.7; partial correlation coefficient 0.6, when considering such factors as severity of dermatitis and coexisting asthma or allergic rhinitis). In the patients with normal serum IgE levels, those with a moderate or severe type of dermatitis had a slightly higher number of eosinophils ($p < 0.05$) than those with a mild dermatitis. Such a difference could not be found in the group of patients with a high serum IgE level. No significant differences in the number of eosinophils between those with "pure" dermatitis and the "mixed" group could be found in patients with a normal IgE level. Such a comparison could not be done in the group with high serum IgE levels as here only one patient had a "pure" dermatitis.

The mean value of circulating basophils did not differ significantly between patients who had a high serum IgE value and those with a normal one ($24/mm^3$, SD 21.2 and $16.6/mm^3$, SD 12.8 respectively). Nor was there any correlation between the patient's IgE value and the number of circulating basophils.

TABLE VII. Comparison between case history of symptoms and intradermal test reactions to thirteen allergens. The figures indicate the number of tests.

	Case history positive		Case history negative	
	High IgE	Normal IgE	High IgE	Normal IgE
Skin test positive	56	18	54	26
Skin test negative	23	24	127	191

TABLE VIII. Comparison between case history of symptoms and RAST reactions to thirteen allergens. The figures indicate the number of tests.

	Case history positive		Case history negative	
	High IgE	Normal IgE	High IgE	Normal IgE
RAST positive	47	11	45	10
RAST negative	32	30	136	209

Discussion

A higher number of positive RAST and intradermal tests was observed in patients with a high IgE level than in those with normal IgE values. The difference was especially pronounced for the number of strongly positive reactions. This might very well be expected as the IgE level in serum reflects the amount of circulating reagins. The higher IgE value is likely to reflect not only reagins against a larger number of allergens but also a higher reagin-titer against some of the allergens. A determination of the serum IgE level was therefore found to be of help in predicting pronounced intradermal reactions and thereby making it possible to avoid their occurrence.

In previous studies (40, 14, 41) the comparisons between intradermal tests and RAST had been made with some selected allergens in patients whose main symptoms were asthma or allergic rhinitis. In this study of patients with atopic dermatitis many also had asthma or allergic rhinitis which had usually been less pronounced. Since we have a different selection of patients and also have studied a larger number of various allergens, our figures cannot be directly compared with those of previous reports.

The birch-pollen extract showed the best agreement between the RAST and intradermal tests, as can be seen from Table VI. We do not know if this is due to the quality of the extract or whether we have selected people with an active birch-pollen allergy. In favour of a good quality of the birch extract is the fact that it is known to be the allergen giving the best agreement with intracutaneous and provocation tests in asthma and allergic rhinitis.

The largest discrepancy between the RAST and intradermal tests was obtained with extracts from horse, cow and house dust. They often gave a negative reaction in the RAST but were positive in the skin test. It is worth noting that we have used the same antigen batches in both test methods. It is possible that the patients with this pattern of reaction have reagins only in the skin and not in the serum, or at least circulating in very small quantities. The house dust extract often represents a heterogenous mixture of compounds and it is also possible that here some unspecific factors are responsible for the release of vasoactive substances in the skin. Finally, it is also possible that the coupling of the allergen to the matrix in the RAST procedure is more difficult to accomplish with some antigens. For instance, if the ratio of antigen:total protein content in the extract is small, a lot of unspecific protein will bind to the matrix and might thereby exclude the small, but

important, antigenic fraction from the binding sites.

It is interesting to note that the food extracts tested accounted for a large part of the test-pairs with a RAST-positive reaction and a negative skin test. The RAST reactions graded as 1 might be border-line values and therefore will not be considered. Of the 23 test-pairs with a negative skin reaction and a RAST of strength 2 to 4, eleven tests were positive to food extracts. It is difficult to say whether this would reflect that more food reagins are circulating than fixed to the skin or if it is due to instability of the extracts when used for skin testing. It is also worth mentioning that 15 of the 23 discordant tests occurred in only 3 patients, but we have no explanation to this.

All the strongly positive RAST reactions had a corresponding positive skin test; however, of the strongly positive skin tests, the RAST was negative in three. They occurred in two patients with normal histamine and control tests. It therefore seems less likely that they are due to an increased non-specific reactivity of the skin. One explanation could be that they have no circulating reagins, but a high amount fixed in the skin. The patients were tested in off season for pollen when the RAST reaction can be of low strength (6).

It is difficult to do an overall comparison between the intradermal test and RAST. The result varies between the different allergens and, in some instances, only a few patients accounted for a large part of a discrepancy.

Five of the 9 patients with a "pure" dermatitis had circulating reagins against some common allergens. In two of the nine positive RAST reactions, there was a correlation with the case history. Here also the intradermal test was positive. We still know very little about to what extent one may have circulating reagins which do not cause symptoms, or what the factor may be that determines whether they give rise to allergic manifestations or not. Similar findings in children will be discussed in Part IV.

In the absence of provocation tests the degree of agreement between case history and test results is very difficult to evaluate. This is evident from Table VII and Table VIII where we have also listed the number of tests where a positive case history with symptoms on exposure to an allergen could not be verified in the tests. The mechanical irritation by wool is often interpreted by the patients as an allergy, but, in 17 of the skin and RAST tests, the result was negative despite complaints. A certain number of patients are also likely to overestimate the influence of certain allergens they

know might cause allergic symptoms.

The number of eosinophil leucocytes was significantly higher in our patients with a high serum IgE level. Factors such as severity of dermatitis and coexistent asthma or allergic rhinitis must be considered when investigating for a correlation between serum IgE values and the number of eosinophils. In favour of the possibility that the IgE level and eosinophils are correlated or regulated by the same factor is that the partial correlation coefficient is close to the total ($r=0.6; 0.7$). That, in the group of patients with a normal IgE, those with a severe atopic dermatitis might have a slightly higher eosinophil count than those with a mild dermatitis does not speak against such a possibility since the eosinophils are here within normal limits.

IV. ALLERGEN-SPECIFIC IgE IN CHILDREN

Patients with asthma or allergic rhinitis often have an increased serum IgE level and circulating reagins against common allergens as determined by the RAST method (21, 5, 45). In adult patients with atopic dermatitis without associated asthma and/or allergic rhinitis ("pure" dermatitis) relatively few and weak reagin titers were found (see Part III). The present investigation was undertaken to find out if a child with a "pure" atopic dermatitis and an elevated IgE level is likely to develop symptoms of asthma or allergic rhinitis. Will the raised level persist or could it sometimes be only a temporary phenomenon? We also wanted to analyze against what allergens the reagins were directed. Can reagins specific to common allergens be detected although the IgE value is normal? Finally we wanted to do a one-year follow up in children with a "pure" atopic dermatitis and normal IgE levels.

Material and Methods

Patients. I. The 9 patients with the highest serum IgE values (R.V. $\geqslant +$ 0.4610) and a "pure" atopic dermatitis described in Part II were asked to come for a follow-up study a year later. In eight patients new blood samples were collected, the patients were examined, and a new case history with special regard to allergic manifestations was once more taken.

II. A random selection of 20 patients was made from the children over 3 years of age described in Part II as having a "pure" atopic dermatitis and serum IgE values within normal limits (R.V. $\pm$ 0.2666). A venous blood sample is necessary for the RAST analyses but it is difficult to obtain in children less than 3 to 4 years old. Seventeen patients were able to come a year later and were examined in the same way as those with high IgE levels.

Determination of IgE. The serum IgE concentrations were determined as described in Part II.

RAST analyses. To simplify the RAST experiment described in Part III, a modification using paper discs as the insoluble matrix was used in the

following way: Allergens were coupled to CNBr-activated filter paper (Munktells OOH) discs with a diameter of 5 mm (42, 44). A volume of 0.1 to 0.2 ml of a reference standard in suitable dilutions or serum samples diluted 1/2 and 1/5 were incubated with allergen-paper discs for 3 hours at room temperature without any shaking or tumbling of the tubes. After washing 5 times with saline containing 0.1% Tween 20, 0.2 ml of ^{125}I-labelled anti-IgE representing approx. 60.000 counts per minute was added and incubated over night at room temperature. The excess of labelled anti-IgE was washed away the next day and the activity bound to the paper discs was measured in a gamma scintillation detector.

Allergen extracts. The same batches of allergen extracts were used as in the study of Berg *et al.* (3), except for horse dander (Vitrum AB) and our own preparations of egg whithe and milk. The allergens are listed in Tables IX and X.

Results

The results for children with an initially high IgE value are given in Table IX. The IgE value had dropped remarkably in patient No. 1. In the other 7 patients the IgE value had remained rather unchanged or decreased only slightly. RAST analyses were performed in only seven of the patients as no venous sample could be obtained from patient No. 8 due to her young age. Four of them had circulating reagins against pollen. Two of the patients also had reagins against animal dander and food extracts. Many of the reactions were strongly positive. In two of the patients with positive RAST, symptoms of allergic rhinitis had developed since the time when they were first studied.

The results for the children who, in the first study, had a normal serum IgE value are given in Table X. In three children (Nos. 11, 22, 25) the serum IgE value had risen to a high level. In 4 children (Nos. 12, 16, 21, 24) there was an obvious decrease. The RAST analyses showed that 7 of the 17 patients had circulating reagins against the tested allergens. All three patients whose IgE values now were high, belonged to this group. Four of the patients had reagins against pollen, two against animal dander and two against food extracts.

TABLE IX. Results of serum IgE determinations and RAST analyses in children with atopic dermatitis and initially high IgE values.

Patient No.	Sex	Age in years 1969-70	IgE ng/ml Winter 1969-70	IgE ng/ml Winter 1970-71	RAST analyses (1–7) Horse	Dog	Cat	Cow	Birch	Timothy	Ox-eye daisy	Dandelion	House Dust	Egg white	Fish	Milk
1	F	3	880	123												
2	M	8	1140	802												
3	F	4	1750	2222		1			5					2	5	2
4	M	1.7	3350	4320*	6	4	4	7	6	3	4	4	5	7	2	5
5	M	3	440	310*					6	2						
6	F	6	570	400					5	3						
7	M	8	570	620												
8	F	1.6	400	678	Not done											

* Developed allergic rhinitis.

TABLE X. Results of serum IgE determinations and RAST analyses in children with atopic dermatitis and initially normal IgE values.

Patient No.	Sex	Age in years 1969-70	IgE ng/ml Winter 1969-70	IgE ng/ml Winter 1970-71	RAST analyses (1 – 7) Horse	Dog	Cat	Cow	Birch	Timothy	Ox-eye daisy	Dandelion	House Dust	Egg white	Fish	Milk
9	M	4	260	473												
10	F	14	280	623		1										
11	F	4	240	919					6							
12	M	9	120	44												
13	F	5	110	91												
14	F	4	100	51												
15	F	5	90	117							4	3				
16	M	8	150	56												
17	F	4	170	168	3			3	4							
18	F	9	160	184												
19	M	3	100	87												
20	M	3	270	161					5							
21	M	12	170	50												
22	F	6	280	985									4	1		
23	F	9	270	310												
24	M	9	170	58												
25	F	4	250	774										3		

The atopic dermatitis was still active and of about the same degree as the foregoing year in all the examined patients. In the group with initially normal serum IgE values, none had developed symptoms of asthma or allergic rhinitis during the year.

Discussion

The serum IgE level was fairly unchanged in most patients when determined a year later. An initially high IgE value dropped considerably in one patient and, in three patients with normal IgE values, they had become high. The change in IgE level was not associated with any obvious change in the clinical picture.

Two of the 25 studied children had developed allergic rhinitis during the year. They both belonged to the group with initially high IgE values. These figures are too small to allow any conclusions, but would fit the theory that patients with a high serum IgE level could be more prone to develop asthma or allergic rhinitis. In support of such a theory would also be the fact that we have found few adult patients with a high IgE level and only atopic dermatitis.

None of the children in the group with initially normal IgE values had experienced symptoms of asthma or allergic rhinitis during the year, although the IgE level had become high in three of them. A longer period of observation would be of interest here. It should, however, be remembered that the serum IgE can be looked upon as the sum of all reagins and titers against only a few allergens might not be reflected as an increased IgE level. It is of interest in this connection to note that three of the children with normal IgE values (Nos. 15, 17, 20) had circulating reagins against some of the common allergens tested. None of these three patients had thus far noticed any allergic symptoms when exposed to the allergens in question.

In the absence of a good provocation test, however, it is difficult to evaluate if the reagins might be of importance for eliciting skin symptoms in patients with only dermatitis. We therefore still do not know if a patient might have reagins against an allergen for a certain period before he develops symptoms on exposure.

V. SEASONAL VARIATION OF IgE LEVELS IN SERUM

In patients with pollen allergy an increase of the serum reaginic activity has been demonstrated during the pollination season with different techniques (30, 34). Berg and Johansson studied the IgE levels in serum from children with seasonal symptoms of asthma and/or allergic rhinitis caused by pollen allergy (5). They found significantly lower IgE values before rather than during the season. It is well-known that most patients with atopic dermatitis improve during the summer time and become worse during the winter season. We therefore considered it of interest to study their IgE level during the course of a year.

Material and Methods

In twenty patients with high serum IgE values and sixteen patients with normal values, repeated IgE determinations were performed during one year. The patients were the same as studied previously (Part III). On an average, five IgE determinations were made covering the periods October-December, February-April, end of May-middle of June (mainly birch pollen season), July-August (mainly grass pollen season), and September-October. On every occasion the severity of the patient's atopic dermatitis was graded as described in Part II and symptoms of asthma or allergic rhinitis recorded. All the serum samples were kept frozen at $-20^{\circ}C$ until analyzed. The IgE determinations were all made at the same time using the technique described in Part II.

Results

The mean IgE values for the different periods are given in Table XI. In the course of a year there was no pronounced variation in the serum IgE level in either group. In patients with a normal serum IgE level it was lower in May-June than in February-April ($p < 0.05$) when calculated on the difference between logarithmically transformed values. In patients with a high serum IgE level, it was lower in July-August than in September-October

TABLE XI. Mean serum IgE values during one year in adult patients with atopic dermatitis.

The figures shown are the geometric mean of IgE in ng/ml.

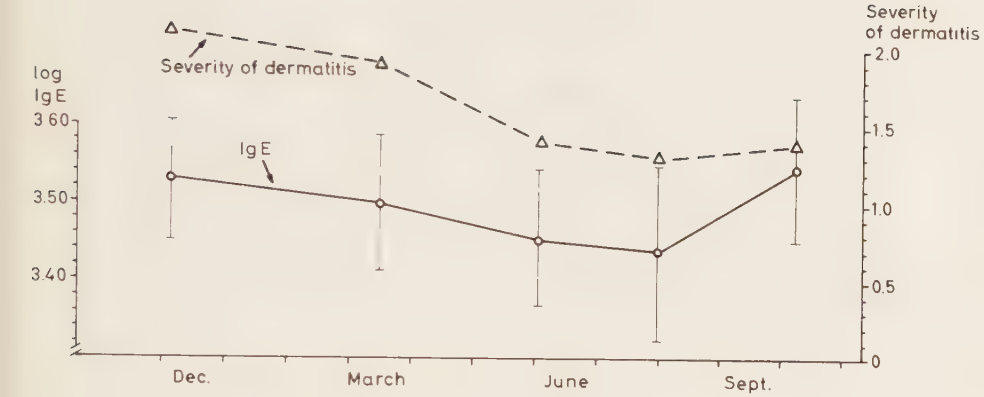
	Patients with high serum IgE values	Patients with normal serum IgE values
October - December	3398	216
February - April	3169	227
May - June	2863	193
July - August	2760	188
September - October	3538	202

($p < 0.01$). No other significant differences were found between the IgE values of two consecutive periods. Neither were the slightly lower IgE values in the summertime significantly different from those taken in the winter. When the mean IgE values were calculated in patients with a positive reaction to pollen on intradermal testing and/or RAST, we did not find any significant change in IgE levels from winter to summertime.

The atopic dermatitis was more pronounced in the wintertime and improved during the summer. As shown in Figures 6 and 7, the degree of severity of atopic dermatitis roughly parallels the IgE curve during the year.

Discussion

In patients with symptoms of asthma or allergic rhinitis elicited by pollen, an increase in the IgE level was reported during or after the pollination season. The rise in pollen-specific IgE was then more pronounced than the increase in the total IgE concentration (6). There is much evidence to believe that the reagins are here involved in the pathogenetic mechanisms. If the same is true for patients with atopic dermatitis, it is more difficult to prove. One reason is the lack of a good provocation test for atopic dermatitis. Indirect evidence, such as a higher serum IgE level in patients with a more severe type of dermatitis (see Part II), would favour a role of the reagins in at least some patients with atopic dermatitis. Another indirect



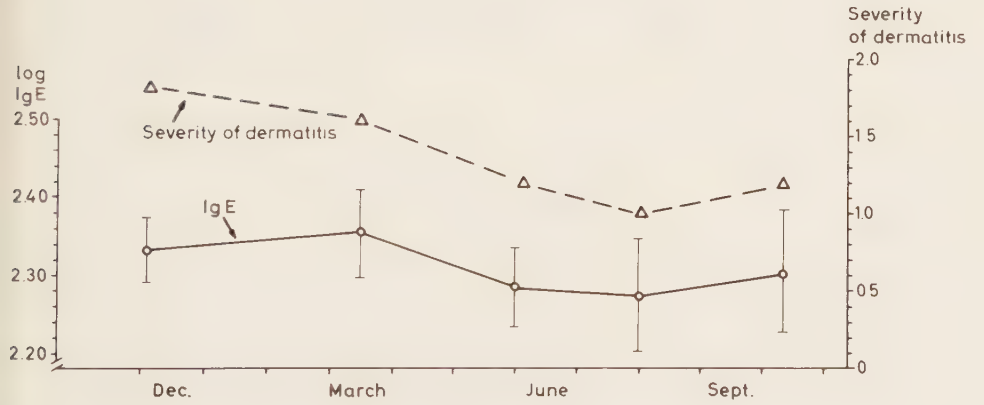


Fig. 6. Means of serum IgE value and of severity of dermatitis during one year in adult patients with atopic dermatitis and high serum IgE values. The vertical lines indicate ± SE of the mean.

Fig. 7. Means of serum IgE value and of severity of dermatitis during one year in adult patients with atopic dermatitis and normal serum IgE values. The vertical lines indicate ± SE of the mean.

evidence would be the findings in this study that the IgE level in serum to some extent paralleled the severity of the dermatitis during the year, but, since the variations in IgE levels were very small, too much emphasis cannot be put hereon. It is interesting to note, however, that even if the patients with a positive test for pollen were considered as a group, they did not show the increase in serum IgE level during the pollination season as reported in patients with allergic rhinitis. On the contrary, they had the same tendency as the entire group to slightly, but not significantly, lower values in the summer. The pollen-specific reagin titers were not determined during the season. It is therefore possible that in some patients an increase of the pollen-specific reagins might occur although it does not influence the total serum IgE level.

VI. SKIN REACTIONS TO ANTI-IgE-ANTIBODY

Specific antibodies of the immunoglobulin E class sensitize human skin for the Prausnitz-Küstner reaction and IgE from a myeloma patient has been found to block passive sensitization with reaginic antibody (18, 39). Quite recently the presence of human IgE on the surface of basophil leucocytes was demonstrated using autoradiography (20). In monkey skin it has been shown with an immunofluorescent technique that the mast cells were the target cells of human ragweed-binding antibodies (16). Ishizaka and Ishizaka discovered that antibodies specific for human IgE (anti-IgE) produced an immediate wheal and flare reaction when injected into normal human skin (19). This urticarial response was thought to be induced by anti-IgE combining with normal IgE fixed to cells in the skin. In a following report, Newcomb and Ishizaka (31) found that the minimum effective dose (MED) of anti-IgE was inversely related to the subject's serum IgE concentration. However, there was no significant difference of MED in non-atopic persons and patients with asthma and allergic rhinitis. Therefore we have studied further the effect of intradermal injections of anti-IgE in patients with atopic dermatitis.

Material and Methods

Patients. Twenty-eight patients, aged 15 to 34 years. Thirteen patients had a mild form of dermatitis, Grade 1 (see Part II), and 15 had more extensive involvment, Grades 2 and 3. A history of asthma or allergic rhinitis was obtained from 22. None of the patients were receiving cortico-steroids, antihistamines or any other drugs. Their serum-IgE values ranged from 80 to 16,200 ng/ml.

Skin tests. A sheep antiserum against Fc-fragments of E protein ND was rendered specific by absorption with normal IgG in solution. The antiserum was diluted until equivalent to 1 mg/ml of IgE(ND) in gel diffusion experiments. The strength of this preparation was called 10°. Serial dilutions 10^{-2}, $10^{-2 \cdot 5}$, 10^{-3}, $10^{-3 \cdot 5}$, 10^{-4}, $10^{-4 \cdot 5}$, 10^{-5}, $10^{-5 \cdot 5}$, 10^{-6}, $10^{-6 \cdot 5}$ were prepared

in saline immediately before each skin testing. Normal sheep serum was used as a negative control. The undiluted serum was called 10°, a dilution of 10^{-2} was used for skin testing.

Intracutaneous injections of 0.10 ml of the test solutions were made through a No. 25G needle on the volar surface of the forearms, if possible on normal looking skin. The size of wheal and flare after 20 minutes was recorded. The dilution of anti-IgE which gave a reaction most closely matching that of 0.10 ml histamine $1:10^{-6}$ was called the minimum effective dose (MED). In most cases this was equivalent to the minimal dose of anti-IgE giving an urticarial reaction. Only one patient showed a positive skin reaction to the sheep control serum and was excluded from the study. The serum IgE values were determined from venous blood samples drawn immediately before the skin testing. The serum was separated and kept frozen until analyzed as described in Part II.

Results

In Figure 8 the MED of the patient has been plotted on a logarithmic scale against the IgE concentration in serum. It is evident from this figure that there is no correlation between these two variables in this study. There was no difference in this respect either between patients with mild or more extensive dermatitis.

Discussion

When our investigation was started no results from similar studies in patients with atopic dermatitis were available. In 1970 Ogawa *et al.* (33) reported that, in 23 patients with atopic dermatitis, it was not possible to distinguish between those with high or low serum IgE levels on the basis of the skin test with anti-IgE antiserum. This is an agreement with our results since we found no correlation between the MED of anti-IgE and the serum IgE level of the patient. Newcomb and Ishizaka's studies were done in normal adults and patients with asthma or allergic rhinitis (31). It is possible that the difference in results might depend on the atopic dermatitis present

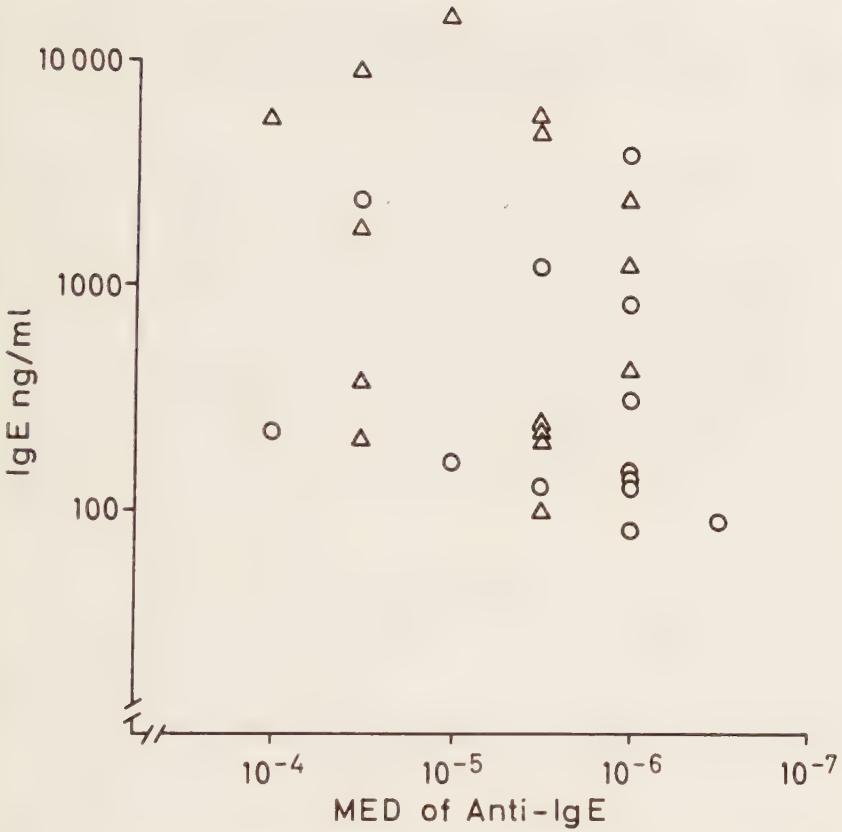


Fig. 8. The minimum effective dose (MED) of anti-IgE giving a standard reaction plotted against the serum IgE in 28 adult patients with atopic dermatitis. O, mild dermatitis; △, moderate-severe dermatitis.

in our patients. The absence of a "halo" around positive skin tests in patients with atopic dermatitis has long been noticed (32). Several of our patients with a high serum IgE level and who required a higher concentration of the anti-IgE serum to elicit the positive reaction displayed some kind of abnormal vascular reaction in the skin as studied in Part VII. It is possible that this abnormality could explain the deviation of their reactions from an expected relationship. Reactions to even dilute solutions of the anti-IgE serum in some of our patients with low serum IgE values could then be explained by a tendency to very pronounced wheal-and-flare reactions. From a comparison with the results obtained in Part VII, we could find no

evidence for this. A more likely possibility would then be that they have a higher concentration of IgE in the skin than in serum.

VII. VASCULAR SKIN REACTIONS IN PATIENTS WITH HIGH AND NORMAL SERUM IgE LEVELS

Abnormal vascular reactions of the skin have been noticed in patients with atopic dermatitis (36 for ref.). Instead of a normal, triple response when firmly stroked, they might show a white dermographism. A reduced flare to histamine and bradykinin and delayed blanching after injections of acetylcholine have also been described. The normal reddening of the skin caused by local application of nicotinic acid esters is often replaced by a blanching phenomenon. The following study was undertaken to investigate whether some of these abnormal vascular reactions were more commonly found in atopic patients with a high rather than those with a low serum IgE level.

Materials and Methods

Patients. I. Twenty adult patients with atopic dermatitis and an IgE level in serum above 1,000 ng/ml.

II. Sixteen adult patients with atopic dermatitis and an IgE level in serum between 125 and 500 ng/ml. Both groups are identical with the ones reported on in Part III.

Drugs used. Histamine hydrochloride, 0.1 mg/ml, sterile solution (Vitrum, Sweden).

Metacholine chloride, 1 mg/ml and 0.2 mg/ml. This was freshly prepared in sterile saline on each day of testing.

Tetrahydrofurfuryl ester of nicotinic acid, 5 per cent in an ointment base (Trafuril, Ciba).

Skin Tests. The dermographic response was studied by firmly stroking the patients forehead and breast with the side of a wooden tongue depressor. The degree of skin involvement varied on the test sites. The stroked area was observed for thirty minutes. The response was considered abnormal if any or both of the tested areas showed a white dermographism during the observation period.

An intradermal injection of 0.01 mg of histamine was made in a skin area on the back which was as little affected as possible. The size of wheal and flare was measured after twenty minutes. The metacholine injections, 0.1 mg and 0.02 mg, were given intradermally on the volar surface of the forearms and, if possible, on seemingly normal skin. The test sites were observed for about thirty minutes.

3 x 3 cm^2 areas in the cubital fold and on the upper arm were rubbed with 0.1 ml of Trafuril ointment. In the cubital folds there were, as a rule, eczematous changes. The area on the upper arm was chosen so as to have a seemingly normal skin. The reactions were studied after 5, 20 and, in most cases, also 60 minutes. Those patients who developed a blanching of the skin in any of the treated areas were considered to have an abnormal response.

IgE Determinations. The IgE determinations were carried out on venous blood samples drawn immediately before testing as has been described in Part II.

Statistical Calculations. The statistical calculations have been done using the Fisher exact probability test.

Results

Histamine Reactions. The results of all the test reactions are listed in Table XII. When comparing the groups with high and low serum IgE levels, there was no significant difference in the number of patients where the histamine flare was weak or absent. When the patient's dermatitis was classified as either mild or moderate-severe (see Part II), all those whose histamine reactions were weak or absent were found in the group with more pronounced symptoms of atopic dermatitis. This difference is significant ($p < 0.01$).

Metacholine Reactions. The delayed blanching phenomenon after metacholine was about as common in all studied groups and no significant differences could be found.

Reactions to a Nicotinic Acid Ester. In eleven patients the abnormal reac-

TABLE XII. Vascular reactions in adult patients with atopic dermatitis with regard to IgE level and severity of dermatitis.

	Histamine flare		Metacholine reaction		Trafuril® reaction		Dermographic response	
	normal	weak or absent	red	delayed blanch	red	blanched	normal	white
High IgE	18	2	16	4	11	7	5	13
Normal IgE	11	5	13	3	6	9	7	9
Mild dermatitis	17	0	14	3	10	7	10	5
Moderate-severe dermatitis	12	7	15	4	7	9	2	17

tion was noted after both five and twenty minutes, but, in five patients, the initial blanching was followed at twenty minutes by a reddening of the skin; these will still be considered abnormal.

No significant difference in the number of abnormal reactions could be seen between patients with high and with normal serum IgE levels. Nor was there any difference with regard to severity of the dermatitis.

Dermographism. There was no significant difference in the occurrence of white dermographism as tested between the patients with high and normal serum IgE values. In the group with a moderate-severe type of dermatitis, the white dermographism was more common ($p < 0.001$) than in those with a mild form.

Discussion

The frequency of abnormal vascular reactions found in patients with atopic dermatitis is, to some extent, dependent on the degree of involvement of the test site (8). The degree of general involvement of the skin and the location of the test area might also influence the reaction. In this study we have, as far as possible, tried to consider these factors when we wanted to determine to what extent the vascular responses might differ in patients with high and low serum IgE levels.

We could not find a significant difference in the number of abnormal

responses between patients with high and normal IgE levels in serum with any of the drugs used. But a reduced or absent flare to histamine was more commonly found in the patients with more pronounced skin involvement when a division was made into groups with mild and with moderate-severe dermatitis. Likewise, a white dermographism was more often seen in those with more extensive skin changes. That no such differences could be found with metacholine might be due to the small number of abnormal reactions. This figure would probably have increased if additional areas had been tested.

On the basis of the results of this study, we can find no correlation between the occurrence of abnormal vascular reactions and the reagin titer of the patients measured as total serum IgE. They seem to be two unrelated phenomena. Champion (8) found a high number of delayed blanching reactions with metacholine in adult patients with atopic dermatitis but no such reaction in patients with atopic asthma and hay fever. This strengthens our findings that the abnormal vascular reactions are not influenced by the reagin level, but instead that they are related to the severity of the dermatitis.

SUMMARY

Reaginic antibodies in serum belong to immunoglobulin E (IgE). The reagins are known to be involved in the pathogenesis of many cases of asthma and allergic rhinitis. In the present investigation IgE was studied in 128 children and 93 adults with atopic dermatitis. Allergen-specific IgE was also studied in selected groups.

Both children and adults with a history of asthma or allergic rhinitis in addition to their dermatitis had a higher mean serum IgE value than the patients with only atopic dermatitis. Most patients in this latter group had a serum IgE value within normal limits. It would thus seem logical to link the finding of a high serum IgE level to the concomitant symptoms of asthma or allergic rhinitis. Patients with more extensive skin changes, however, had a higher mean serum IgE level than those with a mild dermatitis. This could indicate that the reagins might be of importance for eliciting skin changes.

In a selected group of adults, the results of intradermal tests with several common allergens were compared with the occurrence of allergen-specific IgE in serum using a radioallergosorbent technique (RAST). Patients with a high serum IgE level had a higher number of positive test reactions with both methods when compared to those with normal serum IgE levels. The agreement between the two methods, however, depended very much on the allergen tested, but probably also on the selection of patients. The best agreement was found with the birch pollen extract. More than two-thirds of the "one plus" skin reactions had a correspondingly negative RAST. The RAST reaction was positive in 86 per cent of the strongly positive ("three plus") skin tests. A strongly positive RAST reaction (5 to 7) always had a correspondingly positive skin test. The agreement with a positive case history was about the same both for the intradermal tests and RAST, but these figures are uncertain in the absence of a good provocation test for atopic dermatitis.

In children with only atopic dermatitis, we reinvestigated a year later nine with a high serum IgE value and seventeen with a normal serum IgE value. Symptoms of allergic rhinitis during the year were seen in two of the children with initially high serum IgE values. Circulating reagins against common allergens could be demonstrated with RAST in the children with both high and normal serum IgE levels. This was also true for adults with only atopic dermatitis.

In 36 adult patients with high and normal serum IgE levels, the variation of total IgE in serum was followed during a year's time and it was found to be very small. There was no tendency to an increase during the pollination season as had been reported in patients with only asthma or allergic rhinitis from pollen. On the contrary, there seemed to be a slight, although not significant, decrease during the summer and the serum IgE curve during the year roughly paralleled the severity of dermatitis curve.

No correlation was found between the number of circulating basophils and the serum IgE concentration. The eosinophil leucocytes were increased in patients with a high serum IgE level. Here a slight correlation was found between the number of eosinophils and the serum IgE level which indicates a possible relationship.

The discrepancy found between intradermal tests and RAST might be due to a difference in the IgE concentration in the skin and the serum. In an attempt to estimate the approximate IgE level in the skin, we studied the dose-response to intradermally injected anti-IgE antibody. The occurrence of abnormal vascular responses to histamine, metacholine and a nicotinic acid ester, as well as the dermographism to stroking, were also studied in these patients. We found no correlation between the dose of anti-IgE required for a standard skin reaction and the serum concentration of IgE. This might be due to a real difference in the IgE concentration between the serum and the skin or that the response of the vessels of the skin to the antigen-antibody reaction is the dominating factor. An increased vasoconstrictor tendency could possibly explain why some patients with elevated serum IgE levels required a higher anti-IgE dose than expected. We found no support for an increased vasodilator response in the patients requiring only dilute amounts of anti-IgE to elicit the reaction. Patients with a severe dermatitis had a pale reaction to histamine and a white dermographism more often than those with a mild dermatitis. The serum IgE level did not seem to influence the abnormal vascular response as no difference could be found between patients with high or normal serum IgE levels.

ACKNOWLEDGMENTS

I wish to express my gratitude and appreciation to:

My advisors, Professor Lennart Juhlin and Associate Professor S. Gunnar O. Johansson, and to Dr. Tony Foucard and Associate Professor Gerd Michaëlsson, all of whom have provided me with many fruitful discussions and much helpful criticism,

Associate Professor Claes Högman for his kind interest and for placing the facilities of his group at my disposal,

Britt Ytterberg, Lilian Posibiloch and Monica Hagström of the Blood Center, and Irma Kihlman and Christel Wretman of the Dermatology Department for their excellent technical assistance, and

Göran Engholm, M.A., for assistance with the statistical calculations.

This research was supported by grants from the Medical Faculty of the University of Uppsala and the Jörgen Schaumann Foundation.

REFERENCES

1. Axen, R., Porath, J. & Ernback, S.: Chemical coupling of peptides and proteins to polysaccharides by means of cyanogen halides. Nature, 214:1302, 1967.
2. Bennich, H. & Johansson, S.G.O.: Structure and Function of Human Immunoglobulin E. Adv. in Immunol. 13:1, 1971.
3. Berg, T., Bennich, H. & Johansson, S.G.O.: In vitro Diagnosis of Atopic Allergy. I. A Comparison between Provocation Tests and the Radio-allergosorbent Test. Int. Arch. Allergy, 40:770, 1971.
4. Berg, T. & Johansson, S.G.O.: Immunoglobulin Levels During Childhood, With Special Regard To IgE. Acta Paediat. Scand., 58:513, 1969.
5. Berg, T. & Johansson, S.G.O.: IgE Concentrations in Children with Atopic Diseases. Int. Arch. Allergy 36:219, 1969.
6. Berg, T. & Johansson, S.G.O.: In Vitro Diagnosis of Atopic Allergy. IV. Seasonal Variations of IgE Antibodies in Children Allergic to Pollens. A Study of Nontreated Children and of Children Treated with Inhalation of Disodium Cromglycate. Int. Arch. Allergy, in press.
7. Berg, T. & Johansson, S.G.O.: To be published.
8. Champion, R.H.: Abnormal Vascular Reactions in Atopic Eczema. Brit. J. Derm. 75:12, 1963.
9. Coca, A.F. & Cooke, R.A.: On the classification of the phenomena of hypersensitiveness. J. Immunol. 8:163, 1923.
10. Coca, A.F. & Grove, E.F.: Studies in hypersensitiveness. XIII. A study of atopic reagins. J. Immunol. 10:455, 1925.
11. Coca, A.F. & Sulzberger, M.B.: in Yearbook of Dermatology and Syphilology, p. 59, Yr. Bk. Pub., Chicago, 1933.
12. Coca, A.F., Walzer, M. & Thommen, A.A.: Asthma and Hay Fever. Thomas, Springfield, Ill., 1931.
13. Coombs, R.R.A., Hunter, A., Jonas, W.E., Bennich, H., Johansson, S.G.O. & Panzani, R.: Detection of IgE(IgND) Specific Antibody (Probably Reagin) to Castor-Bean Allergen by the Red-Cell-Linked Antigen-Antiglobulin Reaction. Lancet, i:1115, 1968.
14. Fagerberg, E. & Wide, L.: Diagnosis of Hypersensitivity to Dog Epithelium in Patients with Asthma Bronchiale. Int. Arch. Allergy, 39:301, 1970.

15. Glaser, J.: Allergy in Childhood. Thomas, Springfield, Ill., 1956.
16. Hubscher, T., Watson, I.J. & Goodfriend, L.: Target Cells of Human Ragweed-Binding Antibodies in Moneky Skin. J. Immunol. 104:1196, 1970.
17. Ingelfinger, F.J., Lowell, F.C. & Franklin, W.: Gastrointestinal allergy. New Eng. J. Med., 241:303, 1949.
18. Ishizaka, K., Ishizaka, T. & Hornbrook, M.M.: Identification of gamma E-antibodies as a carrier of reaginic activity. J. Immunol. 99:1187, 1967.
19. Ishizaka, K. & Ishizaka, T.: Reversed-type allergic skin reactions by anti-γE globulin antibodies in humans and monkeys. J. Immunol., 100:554, 1968.
20. Ishizaka, K., Tomioka, H. & Ishizaka, T.: Mechanisms of Passive Sensitization. I. Presence of IgE and IgG Molecules on Human Leucocytes. J. Immunol. 105:1459, 1970.
21. Johansson, S.G.O.: Raised Levels Of A New Immunoglobulin Class (IgND) In Asthma. Lancet, ii:951, 1967.
22. Johansson, S.G.O.: Serum IgND Levels in Healthy Children and Adults. Int. Arch. Allergy 34:1, 1968.
23. Johansson, S.G.O. & Bennich, H.: Immunological studies of an atypical (myeloma) immunoglobulin. Immunology, 13:381, 1967.
24. Johansson, S.G.O. & Bennich, H.: Studies on a new class of human immunoglobulins. I. Immunological properties. Nobel Symposium 3, Gamma Globulins, Structure and Control of Biosynthesis, p. 193, Almqvist & Wiksell, Stockholm, 1967.
25. Johansson, S.G.O., Bennich, H. & Berg, T.: Vitro Diagnosis of Atopic Allergy. III. Quantitative Estimation of Circulating IgE antibodies by the Radioallergosorbent Test. Int. Arch. Allergy, in press.
26. Johansson, S.G.O., Bennich, H., Foucard, T. & Lundkvist, U.: Cross-reacting heterophile antibodies to gammaglobulins in normal human serum. Paper presented at the Meeting of the Scandinavian Society for Immunology, Aarhus, 1970. Acta path. microbiol. Scand. (in press).
27. Johansson, S.G.O., Bennich, H. & Wide, L.: A new class of immunoglobulins in human serum. Immunology, 14:265, 1968.
28. Johansson, S.G.O. & Foucard, T.: Personal communication.
29. Juhlin, L., Johansson, S.G.O., Bennich, H., Högman, C. & Thyresson, N.: Immunoglobulin E in Dermatoses. Arch. Derm. 100:12, 1969.

30. Lichtenstein, L.M., Norman, P.S. & Conell, J.T.: Comparison between skin-sensitizing antibody titers and leucocyte sensitivity measurements as an index of the severity of ragweed hay fever. J. Allergy, 40:160, 1967.
31. Newcomb, R.W. & Ishizaka, K.: Skin reactions to anti-γE antibody in atopic, nonatopic and immunologically deficient children and adults. J. Allergy, 43:292, 1969.
32. Norrlind, R.: Prurigo Besnier (atopic dermatitis): Clinical experimental study of its pathogenesis with special reference to acute infections of the respiratory tract. Acta Derm. Ven. 26:1 (suppl. 13), 1946.
33. Ogawa, M., Berger, P.A., McIntyre, O.R., Clendenning, W.E., Tomioka, H. & Ishizaka, K.: IgE in Atopic Dermatitis. Clin. Research Vol. XVIII No 2:350, 1970.
34. Osler, A.G., Lichtenstein, L.M. & Levy, D.A.: In Vitro Studies of Human Reaginic Allergy. Adv. in Immunol., 8:183, 1968.
35. Pillsbury, D.M., Shelley, W.B. & Kligman, A.M.: Textbook of Dermatology. W.B. Saunders Company, Philadelphia, 1956.
36. Rook, A., Wilkinsson, D.S. & Ebling, F.J.G.: Textbook of Dermatology p. 229 ff. Blackwell Scientific Publications. Oxford and Edinburgh, 1968.
37. Rothman, S. & Bloom, R.E.: The increased Vasoconstrictor Tendency in Atopic Dermatitis. Arch. Belg. Derm. et Syph. 13:300, 1958.
38. Shelley, W.B. & Juhlin, L.: Functional Cytology of the Human Basophil in Allergic and Physiologic Reactions: Technique and Atlas. Blood, 19:208, 1962.
39. Stanworth, D.R., Humphrey, J.H., Bennich, H. & Johansson, S.G.O.: Specific inhibition of the Prausnitz-Küstner reaction by an atypical myeloma protein. Lancet ii:330, 1967.
40. Stenius, B. & Wide, L.: Reaginic antibody (IgE), skin and provocation tests to dermatophagoides culinae and house dust in respiratory allergy. Lancet, ii:455, 1969.
41. Stenius, B., Wide, L., Seymour, W.M., Holford-Strevens, V. & Pepys, J.: Clinical significance of specific IgE to common allergens. I. Relationship of specific IgE against Dermatophagoides spp. and grass pollen to skin and nasal tests and history. Clin. Allergy, 1:37, 1971.
42. Varga, D.J. & Ceska, M.: Characterization of allergen extracts by means of electrofocusing and a paper-radio-allergo-sorbent-technique (RAST).

Paper presented at Medicinsk Riksstämma, Stockholm, 1970.
43. Walker, R.B. & Warin, R.P.: The incidence of eczema in early childhood. Brit. J. Derm. 68:182, 1956.
44. Wide, L.E.: Förenklad radioimmunologisk sandwich technique för bestämning av antigener och antikroppar. Paper presented at Medicinsk Riksstämma, Stockholm, 1970.
45. Wide, L., Bennich, H. & Johansson S.G.O.: Diagnosis Of Allergy By An In-Vitro Test For Allergen Antibodies. Lancet, ii:1105, 1967.
46. Wide, L. & Porath, J.: Radioimmunoassay of proteins with the use of Sephadex-coupled antibodies. Biochem. biophys. Acta 130:257, 1966.

UPPSALA OFFSET CENTER AB 1971